The logistics of early embryonic events management to achieve the benefit of PGS

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PGS – aneuploidy screening

…. reveals aneuploid embryos having affected all the cells as a consequence of meiotic errors

…. detects aneuploidies arising *de-novo* as a result of mitotic malsegregations

mitotic errors contribute to the presence of mosaicism which is responsible for some misdiagnosis after PGS

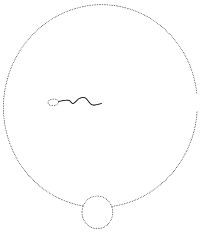
The main tasks for embryologists in PGS cycles

1. to produce as much as possible developmentally competent embryos
2. to identify the embryos with high risk of aneuploidy and mosaicism
3. to keep the viability of embryos unchanged even after invasive intervention without loosing the cells/nuclei for analysis

Ad 1.

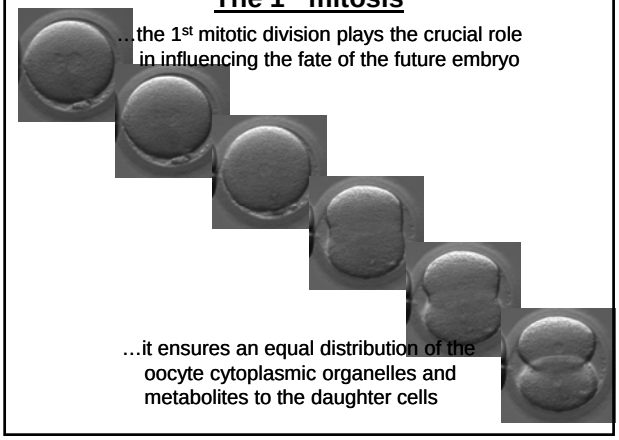
to produce as much as possible developmentally competent embryos…

… ICSI procedure should be preferred for fertilization to obtain standard and high fertilization rate



… can we improve the next embryonic development by the sperm localization during ICSI?

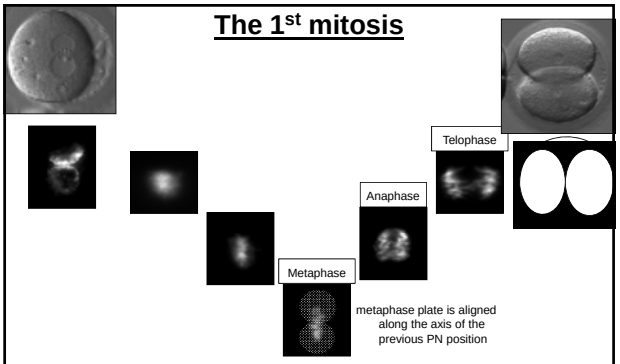
The 1st mitosis



…the 1st mitotic division plays the crucial role in influencing the fate of the future embryo

…it ensures an equal distribution of the oocyte cytoplasmic organelles and metabolites to the daughter cells

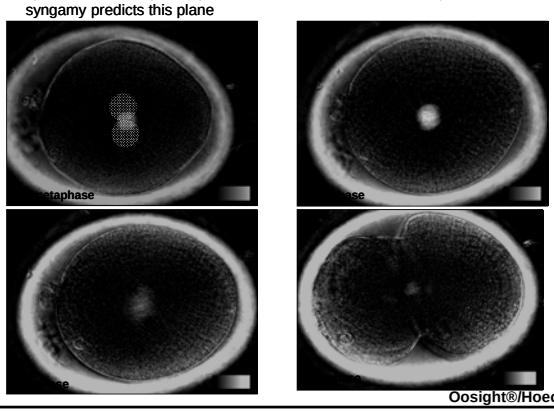
The 1st mitosis



metaphase plate is aligned along the axis of the previous PN position

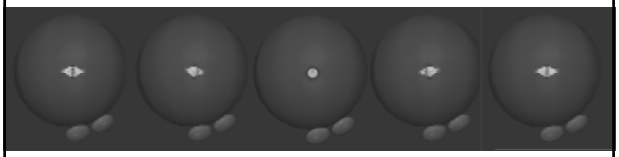
…is responsible for an equal segregation of the chromosomes to daughter cells – the process prerequisite for healthy genetic constitution of early embryos

… it is evident that the 1st cleavage plane is determined by the position of the spindle and corresponding chromosomes and PN orientation just before syngamy predicts this plane



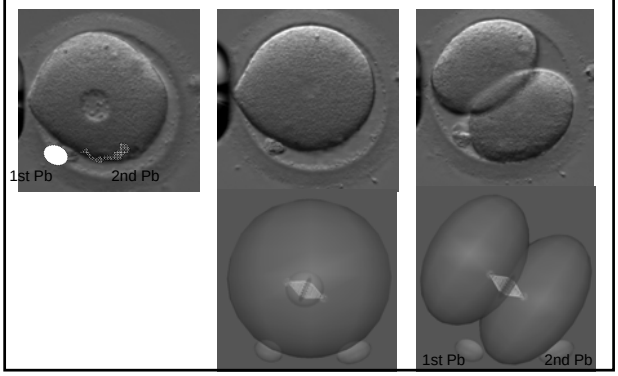
Oosight®/Hoechst

…what determines the poles of the spindle and its spatial orientation?

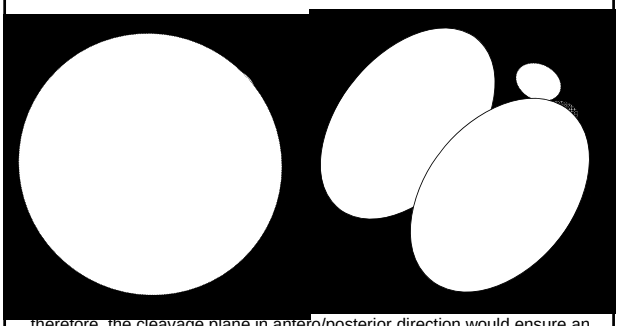


… 3D models of syngamy stage shows the position of the metaphase structure predetermined by previous PN position and its possible spatial orientation

…in the majority of well developing embryos the 1st cleavage plane is oriented towards the 1st Pb (polar body)



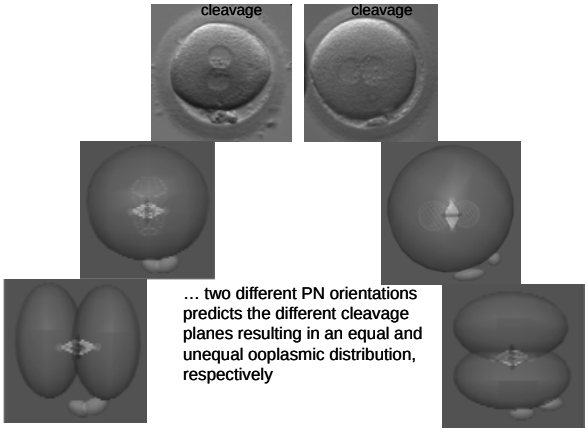
… it is proposed the 1st Pb predicts the animal pole of the oocyte



… therefore, the cleavage plane in antero/posterior direction would ensure an equal distribution of ooplasmic organelles and metabolites to daughter cells of 2cell embryo

Antero/posterior cleavage

Left/right cleavage

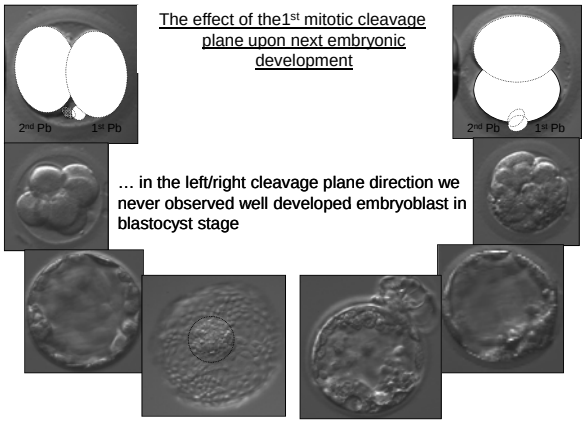


… two different PN orientations predicts the different cleavage planes resulting in an equal and unequal ooplasmic distribution, respectively

Antero/posterior cleavage

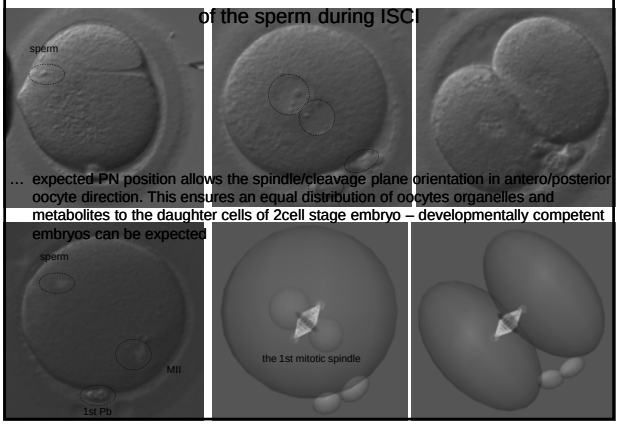
Left/right cleavage

The effect of the1st mitotic cleavage plane upon next embryonic development



… in the left/right cleavage plane direction we never observed well developed embryoblast in blastocyst stage

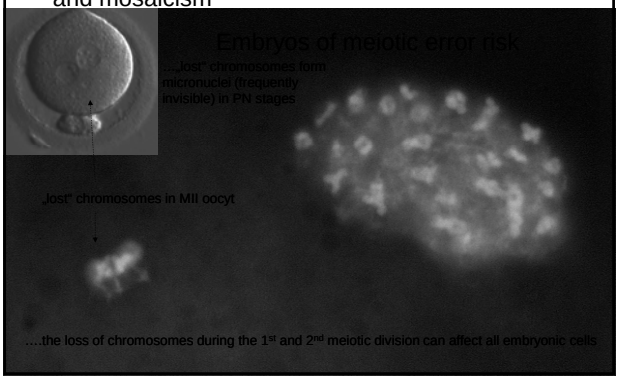
Improvement of embryonic development by the specific deposition of the sperm during ISCI



… expected PN position allows the spindle/cleavage plane orientation in antero/posterior oocyte direction. This ensures an equal distribution of oocytes organelles and metabolites to the daughter cells of 2cell stage embryo – developmentally competent embryos can be expected

Ad 2

to identify the embryos with high risk of aneuploidy and mosaicism



Ad 2

to identify the embryos with high risk of aneuploidy and mosaicism

Embryos of mitotic aneuploidy risk

(based on multinucleation detection)

… the sooner the multinucleations occur the more cells can be altered by aneuploidy

… the first signs of multinucleation should be detected in D1 early cleaved embryos because the correction of multinucleation can occur during the 2nd cleavage

Ad 2

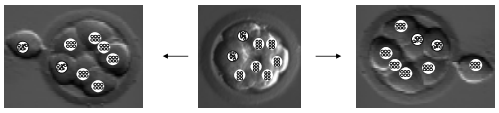
to identify the embryos with high risk of aneuploidy and mosaicism

euploid cells

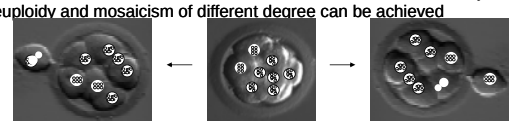
aneuploid cells

Embryos of mosaicism/misdiagnosis risk

(based on daily multinucleation observation)



… the later the multinucleation occur the less cells can be altered by aneuploidy and mosaicism of different degree can be achieved



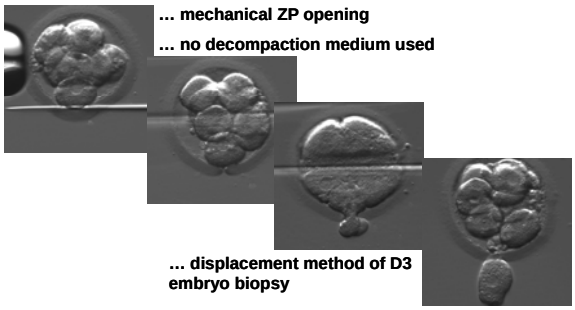
…in accordance with an aneuploid/euploid cells ratio different chance of misdiagnosis can be calculated

Ad 3

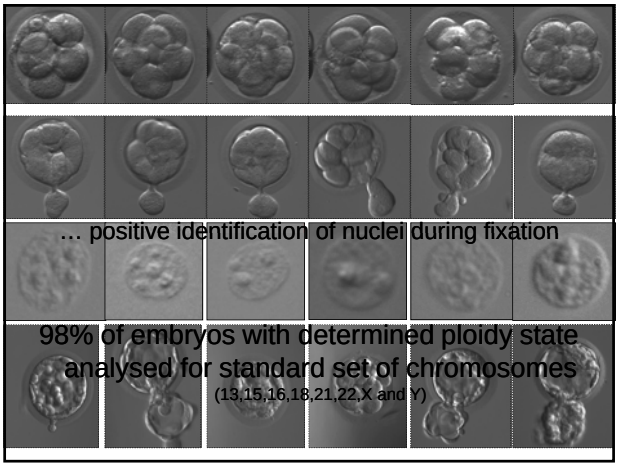
to keep the viability of embryos unchanged even after invasive intervention without loosing the cells for analysis

… mechanical ZP opening

… no decompaction medium used



… displacement method of D3 embryo biopsy



… positive identification of nuclei during fixation

98% of embryos with determined ploidy state analysed for standard set of chromosomes

(13,15,16,18,21,22,X and Y)

Conclusion:

… in order to achieve the benefit of aneuploidy screening in early embryos the biological and technical limitations must be considered and an interactive PGS cycle management is recommended

1. embryology - aneuploidy and mosaicism prediction

reevaluation (3rd round analysis) of questionable findings

2. genetics - FISH aneuploidy detection